Identifying Optimal Biomaterials for Bioscaffolds

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I would like to thank my parents for supporting me through my entire project from start to finish. I would also like to thank Professor Coburn and Elizabeth Van-Zyl for guiding me in the lab, and teaching me methods of working with scaffolds. Finally, I would like to thank my STEM advisor, Ms. Curran, for assisting me from start to finish.

Abstract

Tissue damage is currently one of the leading causes of hospitalization in America and furthermore, the leading cause of surgery throughout the world. Tissue engineering is a developing field that uses healthy tissue around a damaged area to regenerate the damaged cells. The scaffold used when replacing tissue must have mechanical properties similar to that of the original tissue. While many different methods of identifying suitable biomaterials for composing scaffolds exist, there are no existing methods which analyze the properties of these scaffolds and identify the optimal biomaterial. In this project, the mechanical properties of various silk scaffolds were tested, and data was collected. A novel algorithm for identifying optimal biomaterials was then created and tested with sample data. The ability to reasonably identify optimal biomaterials confirmed the success of this project. This research could greatly advance the field of tissue engineering by providing medical experts a reliable method for choosing appropriate biomaterials for tissue repair.

Table of Contents

Literature Review

Tissue Engineering

Tissue injury is a broad term that accounts for many hospital visits annually. These injuries consist of two main types: autonomously reparable injuries, such as dermal and bone injuries, and non-autonomously reparable injuries, such as neural and cartilage injuries. Although autonomously reparable tissues have increased regeneration rates, some cases of severe damage can result in a permanent state of irreparability. For both severe tissue damage and non-autonomously reparable tissue damage, the most widely used method of repairing these tissues is transplants. There are 3 main categories of transplant tissue: autografts, allografts and xenografts. Autografts are tissues that are taken from the patient's own body for transplants. While autografting has advantages, such as high biocompatibility, autografts can be very difficult to obtain, as much of the tissue in a person's body is constantly being used for essential bodily functions. On the other hand, the supply of tissue for allografts, transplantation from human tissue donors, and xenografts, transplantation of animal tissues, is more plentiful, but biocompatibility is lower (Ikada 2006). Tissue engineering, a developing field in biomedical engineering, uses surrounding tissue to grow back the damaged tissue that would have been irreparable otherwise. The process primarily revolves around three integral components: seeded cells, growth factors and cellular scaffolds. Seeded cells are the cells that the body uses to repeatedly divide and make other cells, creating a uniform tissue. Growth factors are used to help the seeded cells develop and mature until the cells are ready to divide. Finally, cellular scaffolds are the framework for the cells to grow on. Cellular scaffolds have many different properties that make them unique and are a major component in tissue engineering. The purpose of these

scaffolds is to model natural extracellular matrices. Extracellular matrices have five main functions: to provide structural support for cells to grow on, to give the tissue its rigidity and flexibility for its basic processes, provide the cues for specific cell functions, to act as reservoirs for growth factors, and provide a degradable environment for morphological changes (Chan 2008).

Mechanical Properties

The mechanical properties of a scaffold are very important in making decisions for whether or not certain scaffolds should be used for tissue engineering. In soft tissue engineering, rigid scaffolds should not be used because they limit movement. Likewise, soft scaffolds would not be suitable for rigid tissue engineering because of the limited compression stress they can withstand without breaking. Generally, synthetic scaffolds with similar mechanical properties as their naturally occurring tissue work best for tissue engineering. As a result, the focus of tissue engineering has gone towards trying to make the mechanical properties of synthesized scaffolds similar to naturally produced extracellular matrices. Currently, there is no perfect method of controlling the mechanical properties of these scaffolds. However, different combinations of biomaterials and methods of synthesis make it possible to create synthetic scaffolds with similar mechanical properties to those of natural extracellular matrices.

There are six main ways of categorizing scaffolds based on their mechanical properties. The first, external geometry, defines the shape of the scaffold, governing aspects such as the physical area of regeneration. The second, surface properties, assist the movement of extracellular molecules. The third, pore size, controls the size of cells that are able to grow in the scaffold, limiting cells from growing too big or staying too small. The fourth, interface adherence, controls how well the scaffold sits in its environment. The fifth, degradation

character, regulates how easily the scaffold degrades once the cells grow back. The sixth and final important mechanical characteristic for all scaffolds is the mechanical competence of the scaffold, which includes mechanical properties such as elastic modulus and tensile strength (Bharatheeswaran 2011). When deciding whether to use a scaffold for medical purposes, these six properties are tested, compared to natural tissue samples, and a decision is made on whether to use the scaffold.

Elastic Modulus

One of the most important mechanical properties of a material is its elastic modulus. Elastic modulus is defined as an object's ability to resist physical deformations. In tissue engineering, the elastic modulus is one of the most important properties of the scaffold, and is a deciding factor in whether or not a synthesized scaffold should be used in a certain situation. As stated before, soft tissue engineering requires a relatively high elasticity in order to maintain dynamic movement, while hard tissue engineering requires low elasticity, in order to maintain rigidity (Diekman 2012). The elastic modulus of a material can generally be calculated by finding the slope of the stress versus strain curve. The stress that an object experiences is defined as the force being applied on that object, while the strain of the object is defined as the change in the ratio of some parameter, such as length, when a certain stress is applied.

In addition, there are three main different types of elastic moduli- Young's modulus, shear modulus and the bulk modulus. The first, Young's modulus, describes tensile elasticity. This measures the elasticity when an object is deformed on a single axis by opposing forces. The shear modulus is similar to Young's modulus in that the force deforms the object into a constant volume, but not necessarily on the same axis. Finally, the bulk modulus is the tendency of an

object to deform in all different directions, therefore changing the volume of the object. The mechanical properties of a biomaterial govern how suitable it is for tissue engineering.

Flexural Modulus

The flexural modulus of an object is defined as the object's tendency to bend. It can also be described as the slope of the stress versus strain graph when discussing flexural deformation. Flexural modulus can be calculated using a method called a three point test, where two ends of an object are placed on supports and a force is applied to the center of the object. The flexural modulus of biomaterials is important for engineering highly flexible places, such as joints. Joints typically require high flexural moduli, as there is almost constantly a three point force being applied (ASTM 2003).

Compressional Strength

The compressional strength of a material is its ability to withstand compressive forces. Every object has an intrinsic compression force limit. Past that limit, the material either breaks or deforms irreversibly. This limiting force is a key feature when synthesizing scaffolds. When dealing with tissue in a high compression environment, such as joints, the scaffold must have a fairly high compressive strength. For example, if a very rigid scaffold with a low compressive limit is used to regenerate a tissue that experiences lots of compressive force, the scaffold has a risk of cracking under potential mechanical stress. Compressional forces are generally used when dealing with malleable materials, as brittle materials generally cannot withstand much compressive force. Their compressive limits are nearly negligible when compared to a malleable material. Therefore, instead of compressional strength, the tensile strength is used to measure the mechanical properties of a brittle material. The compressional strength of an object is a key property used for determining how fit a scaffold is for soft tissue engineering (AZoM, 2016).

Tensile Strength

The tensile strength of a material is its ability to withstand tension forces, as opposed to compressional strength, which is the ability of a material to withstand compressional forces. Tensile strength is commonly used in the analysis of brittle materials because, for brittle materials, the compressional forces are on a significantly lower magnitude compared to malleable materials. Soft tissues are not suitable for tensile strength tests, as they end up ripping due to the tensional forces. These tension force limits greatly affect which synthesized scaffold should be used for the regeneration of certain tissues. For example, in joints, areas that extend more should have a high tension force limit. These forces can rise up to values as high as 170 kg/m^3 (Bullough, 1970). If a high tension force limit is not used, the tissues in that area will rip, leading to more tissue damage in the body. (NDT n.d.)

Average Pore Size

The pore sizes of the scaffolds greatly affect the regeneration rate of the cells inside the scaffold as well. The porosity in the scaffold is arguably the most important feature that a scaffold has. Without a high porosity, there would be no interconnected pathways for cell growth. However, with a high pore density, the scaffold becomes too weak, brittle and unfit for application. The pore size largely determines how slow or fast the cells grow in the scaffold. The pores in the scaffold must be able to comfortably fit the cells inside, without restricting their growth, but at the same time must be small enough to be able to hold the cells in place. Pore size can be measured in many ways, the most common of which is simply looking at the synthesized scaffold under a microscope and estimating the size (Chan, 2008). Pore size is a very important property that governs how cells grow inside a scaffold.

Biomaterials

The biomaterials that compose cellular scaffolds largely define the mechanical properties of the scaffolds. The biomaterial must have a few key properties in order to regenerate damaged tissue without causing other bodily harm. Firstly, the materials must be biocompatible. Tissue engineering is favored over transplants mainly because of biocompatibility, as transplants may cause the body to reject foreign tissue. Similarly, in tissue engineering the scaffold must be biocompatible, otherwise the body would reject the synthesized scaffold in the same way it would reject transplanted tissue. However, biocompatibility of materials is much easier to achieve than biocompatibility of entire tissues. Another important feature required is biodegradability. This feature is important because once the cells grow back in the scaffold, the structure must break down to save space for the natural extracellular matrix to grow. If the scaffold is non-bioresorbable, the cells could get crushed by the residual scaffold. The final aspect that scaffolds must have is a similar set of mechanical properties as a natural extracellular matrix of normal tissue. For example, if the damaged tissue was flexible before damage, a rigid scaffold would lead to reduced mobility. Similarly, in an area of hard tissue growth, such as bone tissue, a soft scaffold could lead to possible damage (O'Brien 2011).

There are two main classes of biomaterials used to make scaffolds: natural polymers and synthetic polymers. The first, natural polymers, are derived from natural sources. This group of biomaterials consists of collagen, elastin and other materials found in natural extracellular matrices. Natural polymers generally have high biocompatibility and biodegradability, but the mechanical properties of these materials are hard to predict. On the other hand, synthetic polymers, such as polylactic acid (PLA) and phosphoglyceric acid (PGA), can be crafted such that they express the desired mechanical properties, but they are rarely biocompatible and

biodegradable (Nigam 2014). Because of this duality in the properties of the biomaterials, a mixture of synthetic and natural polymers is used in medical practice (Chan 2008).

Silk proteins

Soft tissue engineering is one of the most focused areas of tissue engineering study. Damaged soft tissue generally autonomously regenerates at much slower rates than hard tissues. Take neural tissue for example. The regeneration rate of neural tissue is so long that when damaged, neural tissue may not regenerate in an entire lifetime. Another difficulty of soft tissue engineering is the flexibility that the synthesized scaffold must have in order to keep the growing tissue flexible. Silk proteins have emerged as versatile and cheap biomaterials that can be used for tissue engineering, as they have many properties that make them favorable in tissue engineering. These natural proteins are generally very compatible with cells, reducing the risk of the host body rejecting the scaffold. Also, for the same reasons, silk is biodegradable- another advantage of using it for tissue engineering.

Many methods of synthesis have been used to make silk scaffolds, such as solvent casting and particulate leaching, gas foaming, and lyophilization (freeze drying). The most common of these is solvent casting and particulate leaching (SCPL), because it creates a sturdy scaffold suitable for bone tissue engineering. However, for soft tissue engineering, lyophilization techniques become a much better option than solvent casting and particulate leaching. Silk lyophilization techniques make it easy to control the mechanical properties of the synthesized scaffold. Various alterable properties of silk proteins allow for the mechanical properties of silk scaffolds to be controlled. For example, the molecular weight of the silk protein can be easily altered based on the time spent boiling an original silk solution. Also, the concentration of the

silk solution can change the mechanical properties of the synthesized scaffold. Overall, silk is a very versatile biomaterial suitable to be used for tissue engineering (Rnjak-Kovacina 2015).

Collagen

Collagen is one of the most common biomaterials currently used for creating scaffolds. Collagen is the most common protein in extracellular matrices. To date, 28 different types of collagen have been found. Of these 28 different types, Collagen type 1 has been most commonly used for creating synthetic scaffolds. Firstly, since it is the most abundant protein in natural extracellular matrices, collagen is generally biocompatible with human tissues, reducing the risk of body rejection. Another major advantage of using collagen for scaffolds is that collagen fibers can easily become incorporated into the natural extracellular matrices created by the seeded cells. However, collagen does has its own drawbacks. Its mechanical properties prove to be too flexible for rigid tissue repair. However, this has been solved with other methods. Another important property of collagen that makes it a versatile biomaterial is its ability to mix with many substances fairly well. As a result, the weak and overly-flexible collagen can be strengthened by combining it with other biomaterials. The most common biomaterials combine with collagen are glycosaminoglycans (GAGs). GAGs are long polysaccharides created from the repeated linkages of the same single unit disaccharide. However, these collagen-GAG scaffolds still lack the mechanical integrity similar to that of natural extracellular matrices, and therefore a lot more work needs to be done until a perfect collagen-GAG scaffold is created (Nigam, 2014).

Scaffold Synthesis

The methods used to synthesize scaffolds greatly affect the mechanical properties of the scaffolds. There have been countless techniques used to generate cell scaffolds, such as freeze drying (lyophilization), electrospinning, solvent casting and particulate leaching, and self-

assembly. Different synthesis techniques are required, depending on the chemical properties of the biomaterials themselves.

Freeze Drying

Freeze drying, which is also known as lyophilization, is a very common technique used to make solid biomaterial sponges and scaffolds. Freeze drying greatly reduces the temperature of the biomaterial causing it to harden. Then, the pressure inside the lyophilization chamber is greatly reduced, until the water in the solution being lyophilized sublimes into water vapor, leaving the scaffold. This process generates a solid solute scaffold with a highly porous interior complex, which can be used to grow cells.

Electrospinning

Electrospinning takes advantage of nanofibers to create the scaffold. Electrospinning starts with a solution containing the desired biomaterial inside a syringe. A tiny drop of the solution is carefully squeezed out until a drop forms on the tip of the needle. This requires that the solution have a fairly high surface tension for the drop to form. Once the drop is formed, a high voltage current is passed through the needle. The drop then slowly starts to form into a cone shape, with the tip of the cone pointing away from the syringe. As the voltage is increased, the tip of the cone gets thinner and longer, creating a microfiber. Microfibers are then collected on a rotating apparatus. Overlaying the nanofibers creates a highly porous and complex scaffold for the cells to grow in. Electrospinning techniques create scaffolds most similar to natural extracellular matrices (Vasita 2006).

Solvent Casting and Particulate Leaching

For solvent casting and particulate leaching, a solution is made containing the biomaterial and a soluble particle, such as salt (NaCl). Once the particles are all fully dissolved, the new solution is poured into a cast of the required shape. Then, the solution is hardened, causing the

solvent and the particulates to solidify. Next, the entire scaffold is thoroughly rinsed to leach the particulates out. This method is one of the most commonly used methods for generating scaffolds, because it is the cheapest and requires little expensive technology.

Conclusion

Many different properties of cellular scaffolds affect the regeneration rates of cells in the healing tissue. These properties include the biomaterial composition, such as silk or collagen, which accounts for features such as the biodegradability and biocompatibility of the scaffolds, and the method of synthesis, which changes key properties such as the porosity of the scaffold. Although there are a wide variety of options for scaffold synthesis, no “best” option has been created for medical use. Finding the best scaffolds for particular uses is still a problem being researched+ in the field of tissue engineering. Overall, tissue engineering is a growing field with many unexplored and potentially groundbreaking discoveries.

Overview of Research Plan

Phrase 1

While many different methods of identifying suitable biomaterials for composing scaffolds exist, there is no centralized database capable of analyzing the properties of these scaffolds and identify the optimal biomaterial.

Phrase 2

The goal was to engineer a program which successfully identifies the optimal biomaterials to use for scaffolds when repairing tissues.

Overview of Research Methods

Silk Scaffold Synthesis

To make the silk scaffolds, various concentrations of silk (1%, 2%, 3%, 4%, 5% and 6%) were made. Then, the various solutions were lyophilized (freeze dried) to solidify the resulting product. The solid products were then placed in a vacuum chamber until ready for further use. The products were then autoclaved, insolubilizing the products so that they would not dissolve once rehydrated. Finally, the products were placed water for rehydration.

Chicken Decellularization

To generate a natural extracellular matrix to compare with the synthesized scaffold models, a chicken drumstick was bought from the grocery store. Chicken tissue was used because it was a cheap and effective way of modelling human muscle tissue without requiring the potential biohazards of working with live human cells. The chicken samples were then completely decellularized, leaving behind an extracellular matrix.

Mechanical Testing

For mechanical testing, a universal testing machine was used. The machine gave readings of displacement and load, which were converted to strain and stress respectively.

Database Creation

To create the database, a MySQL database was created, containing information on both mechanical information and user information. A program was created in which a user could enter mechanical testing data they collected, or could input natural tissue data and receive the optimal biomaterial for creating scaffolds. A login system was also created as well, such that user data could be saved with the mechanical testing data.

Materials

Material	Use	Supplier
6.15% stock silk solution	Stock solution for making other concentration solutions	Coburn Lab
Centrifuge tubes	Storage	Coburn Lab
24 well plate	Lyophilization	Coburn Lab
Sharpie	Labelling	Coburn Lab
Lyophilizer	Lyophilization	Coburn Lab
Vacuum Chamber	Storage	Coburn Lab
Autoclave	Insolubilization	Coburn Lab
Razor blade	Cutting	Coburn Lab
Deionized Water	Hydration	Coburn Lab
Chicken Leg	Used to obtain muscle tissue samples	Trader Joes
Scalpel	Cutting	Coburn Lab
Sodium Dodecyl Sulfate	Decellularization	Coburn Lab
Aspirator	Aspirating fluids	Coburn Lab
Biopsy Punch	Used for biopsy punching samples	Coburn Lab
Petri Dishes	Storage	Coburn Lab
Universal Testing Machine	Mechanical Testing	Goddard Hall Lab 007

Methods

Data Collection

To start, a 6.15% solution of silk was produced and provided by Coburn Lab. 5mL of six different concentrations, 1%, 2%, 3%, 4%, 5% and 6% were created in centrifuge tubes. 1mL of each solution was pipetted from the centrifuge tube into a well of a 24 well plate. This was repeated 4 times for each concentration. The 24 well plate was then labelled with a sharpie to know which concentrations were in which wells. The 24 well plate was then placed in a lyophilizer by a trained lab specialist, and the silk solutions were lyophilized to make 24 scaffolds, four of each concentration. The scaffolds were stored in a vacuum chamber until ready for next step. The scaffolds were then placed in an autoclave by a trained lab specialist, making the scaffolds insoluble. The insolubilized scaffolds were then placed in water for rehydration. Finally, the scaffolds were then cut to a height of 3mm using a razorblade.

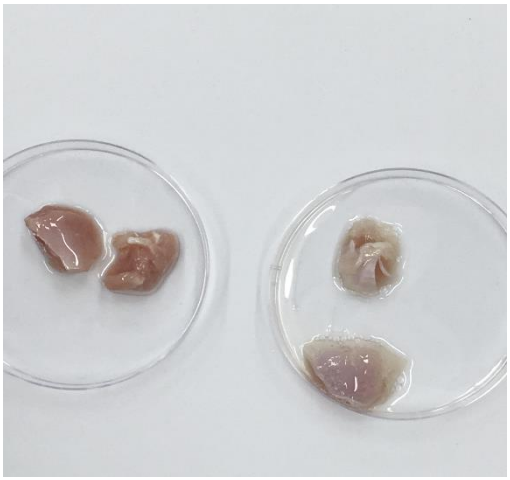

Figure A: Chicken muscle samples. Left: Untouched muscle tissue. Right: Partially decellularized muscle tissue.

While preparing the scaffolds, muscle tissue samples were created. To make these muscle tissue samples, a regular chicken leg piece was taken from the grocery store. Then, using a scalpel, multiple tissue samples of approximately 10mm in diameter and 3mm high were cut from the leg piece. 30ml of a 1% SDS solution were made in a centrifuge tube, and the tissue samples were placed in as well. The centrifuge tube was then attached to a shaker using tape, and shaken at 25% speed for roughly five days. The centrifuge tube was then removed from the shaker, and the liquid inside the centrifuge tube was aspirated, leaving only decellularized chicken tissue behind.

All samples, scaffolds and decellularized chicken tissue, were taken out of their centrifuge and placed on separate petri dishes. Then, each sample was biopsy punched using a 3 mm diameter biopsy puncher. The biopsy punched samples were placed in separate centrifuge tubes containing approximately 5mL deionized water, and the remaining materials were all discarded.

To test the mechanical properties, the biopsy punched samples were placed on a Universal Testing Machine, which gave out readings of Force vs Time. Once all samples were tested, all centrifuge tubes and samples were discarded in red biohazard bags.

Program Creation

A MacOS X High Sierra virtual machine was created on VirtualBox on a Lenovo laptop running Windows 10 (SKU LENOVO_MT_20309). On the virtual machine, Eclipse Oxygen, Apache Tomcat, SequelPro, MySQL and a JDBC jar file were downloaded and installed. A Dynamic Web Project was then created on Eclipse. The JDBC jar file was then placed on the build path of the project, and the Tomcat server was linked to the project. In SequelPro, two databases, one for user information and one for mechanical testing information, were created. Then, multiple different versions of the program were created:

Version I: The program only contained java-based login and account creation functionalities. All inputs and outputs were done in the Eclipse console. User inputs were able to access and modify the database as well as its tables.

Version 2: A web version of Version I was created. This entailed the creation of JSP files to receive user input from submission forms on the webpage. External links to other pages were added as well as simple navigation around the webpages.

Version 3: A data addition portion was added to the web version with the JSPs

Version 4: The material identification portion of the program was added to the previous version.

Results

The data produced by the Universal Testing Machine was in the form of Load (N) vs Displacement (mm). Using this data, and knowing the cross-sectional areas of the samples, the stress and strain values for the data were calculated. The data was then graphed on excel, (*Figure A*) and the Young's Modulus was found by taking the slope of the initial portion of the stress-strain curve. This was repeated for all samples, producing multiple Young's Moduli for each material tested. The tested values on silk were then placed into the database and the program was run. Tested chicken tissue data was then inputted and the program found that 2% Silk modeled the chicken tissue best, with a p-value of 0.4368.

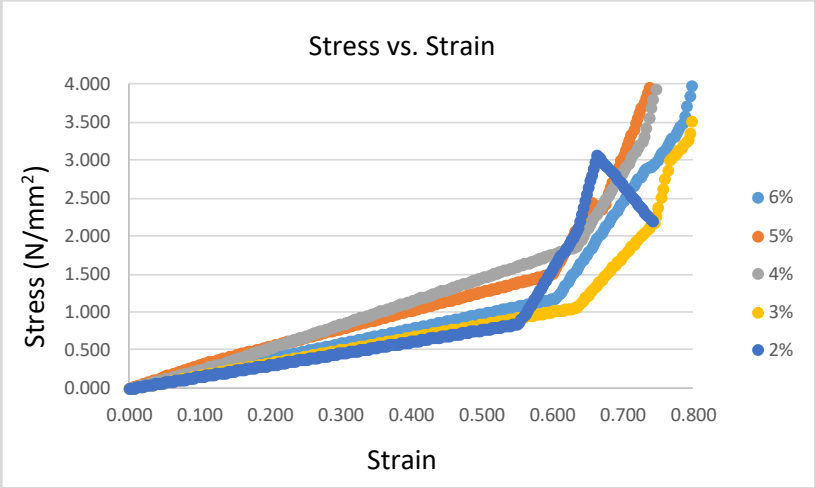

Figure B: Representative data for each of the concentrations of silk used.

Conclusion

Criteria	Weights	Version I	Version II	Version III
Aesthetic Quality	2	4	6	6
Predictive Accuracy	10	0	0	10
Usability	6	5	6	7
Safety	8	7	7	9
Back-End Compatibility	5	2	2	8
	Totals:	104	114	266

Seeing as the highest p-value between the materials in the database and the chicken tissue data was relatively low ($p=0.4368$), it can be reasonably assumed that the materials in the database are not suitable for reengineering muscle tissue on a chicken. This likely resulted from the lack of data in the database. However, as more data is added, more materials with properties similar to that of the chicken tissue will be present, and higher p-values will result. Possible sources of error include differences in hydration levels of the scaffolds when placed on the testing machine, causing inconclusive data in regards to the t-tests performed.

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Appendix A: Limitations and Assumptions

- Time was a major limitation. Because of little time to do the project, the amount of data needed to make reasonable conclusions was not enough
- Money prevented me from being able to buy highly expensive materials, which resulted in me using silk for my experiments.
- I had only a simple degree of authorization for the lab so I was not able to perform all the steps in my procedure by myself
- Machine availability prevented me from using various machines whenever I wanted
- All testing machines gave me accurate results
- All tasks told to authorized lab specialists were done properly
- All concentrations and type of material provided by lab was correctly labelled

Appendix B: Code

Java

```
package Connections;


import java.sql.DriverManager;


import java.sql.*;


public class ConnectionManager {
	public static Connection getConnection() {
		Connection conn=null;
		try {
			Class.forName("com.mysql.jdbc.Driver");

	conn=DriverManager.getConnection("jdbc:mysql://localhost:3306/STEMProject","root",
"password");
			return conn;
		}
		catch(Exception e){
			e.printStackTrace();
			return null;
		}
	}
}
```

```
package Controllers;

import java.io.IOException;
import java.io.PrintWriter;

import javax.servlet.ServletException;
import javax.servlet.annotation.WebServlet;
import javax.servlet.http.HttpServlet;
import javax.servlet.http.HttpServletRequest;
import javax.servlet.http.HttpServletResponse;

import Repositories.UserRepository;

/**
 * Servlet implementation class AccountController2
 */
@WebServlet("/AccountController")
public class AccountController extends HttpServlet {
	private static final long serialVersionUID = 1L;
	UserRepository userRespository;

    public AccountController() {
        super();
		userRespository=new UserRepository();

        // TODO Auto-generated constructor stub
    }
```

```
	protected void doGet(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
//		String username=request.getParameter("username");
//		response.setContentType("text/plain");
//		PrintWriter out= response.getWriter();
//		out.println("<html>");
//		out.println("<head>");
//		out.println("<title>test</title>");
//		out.println("</head>");
//		out.println("<body>");
//		out.println(username);
//		out.println("</body>");
//		out.println("</html>");
	}

	protected void doPost(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub
		if(userRespository.checkUser(request.getParameter("username"))==true) {
			System.out.println("Username is in use. Try another");
			response.sendRedirect("NewAccount2.jsp");
		}
		else {
			userRespository.save(request.getParameter("username"), request.getParameter("password"), request.getParameter("name"), request.getParameter("email"));
			response.sendRedirect("SuccessfulCreationLogin.jsp");
		}

	}
```

```
}
```

```
package Controllers;

import java.io.IOException;
import javax.servlet.ServletException;
import javax.servlet.annotation.WebServlet;
import javax.servlet.http.HttpServlet;
import javax.servlet.http.HttpServletRequest;
import javax.servlet.http.HttpServletResponse;

import Repositories.DataRepository;

/**
 * Servlet implementation class BestScaffoldController
 */
@WebServlet("/BestScaffoldController")
public class BestScaffoldController extends HttpServlet {
	private static final long serialVersionUID = 1L;
	DataRepository dr;

    /**
     * @see HttpServlet#HttpServlet()
     */
    public BestScaffoldController() {
        super();
        dr=new DataRepository();
        // TODO Auto-generated constructor stub
    }
```

```
	/**
	 * @see HttpServlet#doGet(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doGet(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub
		//response.getWriter().append("Served at: ").append(request.getContextPath());
	}

	/**
	 * @see HttpServlet#doPost(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doPost(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		dr.statsTest(request.getParameterValues("myInputs[]"));
	}

}
```

```
package Controllers;

import java.io.IOException;
import javax.servlet.ServletException;
import javax.servlet.annotation.WebServlet;
import javax.servlet.http.HttpServlet;
import javax.servlet.http.HttpServletRequest;
import javax.servlet.http.HttpServletResponse;

import Repositories.DataRepository;

/**
 * Servlet implementation class DataAdditionController
 */
@WebServlet("/DataAdditionController")
public class DataAdditionController extends HttpServlet {
	private static final long serialVersionUID = 1L;
    DataRepository dr;
    /**
     * @see HttpServlet#HttpServlet()
     */
    public DataAdditionController() {
        super();
        dr=new DataRepository();
        // TODO Auto-generated constructor stub
    }

	/**
```

```
	 * @see HttpServlet#doGet(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doGet(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub
		//ResultSet rs=dr.MaterialData();


	}


	/**
	 * @see HttpServlet#doPost(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doPost(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub
		dr.save(request.getParameter("material"), request.getParameter("modulus"));
		response.sendRedirect("DataAddition.jsp");
	}


}
```

```
package Controllers;

import java.io.IOException;
import javax.servlet.ServletException;
import javax.servlet.annotation.WebServlet;
import javax.servlet.http.HttpServlet;
import javax.servlet.http.HttpServletRequest;
import javax.servlet.http.HttpServletResponse;

import Repositories.UserRepository;

/**
 * Servlet implementation class LoginController
 */
@WebServlet("/LoginController")
public class LoginController extends HttpServlet {
	private static final long serialVersionUID = 1L;
	UserRepository userRepository;
	public static String usernameLogin;

    /**
     * @see HttpServlet#HttpServlet()
     */
    public LoginController() {
        super();
        userRepository=new UserRepository();
        // TODO Auto-generated constructor stub
    }
```

```
	/**
	 * @see HttpServlet#doGet(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doGet(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub


	}


	/**
	 * @see HttpServlet#doPost(HttpServletRequest request, HttpServletResponse response)
	 */
	protected void doPost(HttpServletRequest request, HttpServletResponse response) throws ServletException, IOException {
		// TODO Auto-generated method stub
		usernameLogin=request.getParameter("username");
		if(userRepository.userLogin(request.getParameter("username"), request.getParameter("password"))) {
			response.sendRedirect("LoggedIn.jsp");
		}
		else {
			response.sendRedirect("index.jsp");
		}
	}


}
```

```
package Repositories;

import java.sql.Connection;
import java.sql.PreparedStatement;
import java.sql.ResultSet;
import java.sql.SQLException;
import java.sql.Statement;
import java.util.ArrayList;

import Connections.ConnectionManager;
import Controllers.LoginController;

public class DataRepository {
	Connection conn;
	Statement statement;
	ResultSet resultSet;
	ArrayList<String> materialTypes;

	public DataRepository() {
		try {
			conn=ConnectionManager.getConnection();
			statement=conn.createStatement();
		} catch (SQLException e) {
			// TODO Auto-generated catch block
			e.printStackTrace();
		}
	}
	public ResultSet getMaterialTypes(){
```

```
		try {
			resultSet=statement.executeQuery("Select distinct MaterialType from
Mechanical_Data");
		}
		catch (SQLException e) {
			// TODO Auto-generated catch block
			e.printStackTrace();
		}
		return resultSet;
	}
	public String statsTest(String[] moduli) {
		resultSet=getMaterialTypes();
		for(int i=0;i<moduli.length;i++) {
			System.out.println(moduli[i]);
		}
		double[] numModuli= new double[moduli.length];
		double meanInput;
		double stdevInput;
		double sumInput = 0;
		double diffSqInput=0;
		for(int i=0;i<moduli.length;i++) {
			numModuli[i]=Double.parseDouble(moduli[i]);
			sumInput+=numModuli[i];
		}
		meanInput=sumInput/moduli.length;

		for(int i=0;i<moduli.length;i++) {
			diffSqInput+=Math.pow(meanInput-numModuli[i], 2);
		}
```

```
            stdevInput=Math.sqrt(diffSqInput/(double)moduli.length);
            try {
                while(resultSet.next()) {


                }
            }
            catch (SQLException e) {
                // TODO Auto-generated catch block
                e.printStackTrace();
            }
            return "hello";
        }
        public void save(String materialType, String Modulus) {
            try {
                PreparedStatement preparedStatement=conn.prepareStatement("select id
from Mechanical_Data");
                ResultSet idResultSet=preparedStatement.executeQuery();
                idResultSet.last();
                int last=idResultSet.getInt(1);
                System.out.println(last);
                preparedStatement=conn.prepareStatement("insert into
Mechanical_Data(id,MaterialType,YoungsModulus,User) values(?,?,?,?)");
                preparedStatement.setString(1, Integer.toString(last+1));
                preparedStatement.setString(2,materialType);
                preparedStatement.setString(3, Modulus);
                preparedStatement.setString(4, LoginController.usernameLogin);
                preparedStatement.executeUpdate();
            }
            catch(Exception e) {
```

```
                    e.printStackTrace();
                }
            }
        }
```

```
package Repositories;

import java.sql.*;

import Connections.ConnectionManager;

public class UserRepository {
	Connection conn;
	Statement statement;
	ResultSet usernameRs;
	public UserRepository(){
		try {
			conn=ConnectionManager.getConnection();
			statement=conn.createStatement();
		} catch (SQLException e) {
			// TODO Auto-generated catch block
			e.printStackTrace();
		}
	}
	public boolean userLogin(String username, String password) {
		boolean check=false;
		try {
			usernameRs=statement.executeQuery("Select * from UserData");
			while(usernameRs.next()) {

	if(username.equals(usernameRs.getString("Username"))&&password.equals(usernameRs
.getString("Password"))) {
					check=true;
				}
```

```
                }
        }
        catch (SQLException e) {
                // TODO Auto-generated catch block
                e.printStackTrace();
        }
        return check;
}
public boolean checkUser(String username) {
                boolean check=false;
                try {
                        usernameRs=statement.executeQuery("Select * from UserData");
                        while(usernameRs.next()) {
                                if(username.equals(usernameRs.getString("Username"))) {
                                        check=true;
                                }
                        }
                }
                catch (SQLException e1) {
                        // TODO Auto-generated catch block
                        e1.printStackTrace();
                }
                return check;

}

public void save(String username,String password,String name,String email) {
        String userID="failed";
```

```
            try {
                PreparedStatement preparedStatement=conn.prepareStatement("select id
from UserData");
                ResultSet idResultSet=preparedStatement.executeQuery();
                idResultSet.last();
                int last=idResultSet.getInt(1);
                System.out.println(last);
                preparedStatement=conn.prepareStatement("insert into
UserData(id,Name,Username,Password,Email) values(?,?,?,?,?)");
                preparedStatement.setString(1, Integer.toString(last+1));
                preparedStatement.setString(2,name);
                preparedStatement.setString(3, username);
                preparedStatement.setString(4, password);
                preparedStatement.setString(5, email);
                preparedStatement.executeUpdate();
            }
            catch(Exception e) {
                e.printStackTrace();
            }
        }

}
```

JSP

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<%@ page import="java.io.*,java.lang.*,java.util.*,java.net.*,java.util.*,java.text.*"%>
<%@ page import="javax.servlet.http.*,javax.servlet.*,Repositories.*"%>
<%@ page import ="java.sql.*" %>
<title>Data Addition Page</title>
</head>
<body>
<h1>Add Data</h1>
<form method="POST" action="DataAdditionController">
                Material Type: <input type="text" name="material">
                <br>
                Young's Modulus: <input type="text" name="modulus">
                <br>

                <input type="submit" value="submit">
</form>
<% DataRepository dr=new DataRepository(); %>
Current Material Types (Copy EXACTLY if entered data fits one of the below):
<% ResultSet rs=dr.getMaterialTypes(); %>
<% while(rs.next()){
        out.println("<br>"+rs.getString(1));
}
```

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<script src="AddField.js" language="Javascript" type="text/javascript"></script>
<title>Biomaterial Identification</title>
</head>
<body>
<h1>Identify Biomaterials</h1>
<p>
This page helps to identify the optimal biomaterial to use for creating scaffolds for tissue
engineering.

To start, enter mechanical data from the tissue you are trying to replace. Click submit when
finished
</p>
<script src="AddField.js" language="Javascript" type="text/javascript"></script>
<form method="POST" action="BestScaffoldController">
    <div id="dynamicInput">
        Modulus 1: <input type="text" name="myInputs[]">
    </div>
    <input type="button" value="Add another text input" onClick="addInput('dynamicInput');">
         <input type="submit" value="Submit">
</form>
</body>
</html>
```

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<%@ page
    import="java.io.*,java.lang.*,java.util.*,java.net.*,java.util.*,java.text.*"%>
<%@ page import="javax.servlet.http.*,javax.servlet.*,Connections.*"%>
<%@ page import ="java.sql.*" %>

<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<title>Login Page</title>
</head>
<body>

<h1>Login Page</h1>
<form method="POST" action="LoginController">
                Username: <input type="text" name="username">
                <br>
                Password: <input type="password" name="password">
                <br>
                <input type="submit" value="submit">
</form>
<a href="NewAccount2.jsp">Make new account</a>

</body>
</html>
```

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<title>Welcome</title>
</head>
<body>
<h1>Welcome!</h1>
<h2>Would you like to:</h2>
<a href="DataAddition.jsp">Add Data</a>
<br/>
<a href="GetBestScaffold.jsp">Get Best Biomaterial</a>
</body>
</html>
```

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<title>New Account</title>
</head>
<body>
<h1>New Account</h1>
<form method="POST" action="AccountController">
                Name: <input type="text" name="name">
                <br>
                Username: <input type="text" name="username">
                <br>
                Password: <input type="text" name="password">
                <br>
                Email: <input type="text" name="email">
                <br>
                <input type="submit" value="submit">
</form>

</body>
</html>
```

```
<%@ page language="java" contentType="text/html; charset=UTF-8"
    pageEncoding="UTF-8"%>
<!DOCTYPE html PUBLIC "-//W3C//DTD HTML 4.01 Transitional//EN"
"http://www.w3.org/TR/html4/loose.dtd">
<html>
<head>
<meta http-equiv="Content-Type" content="text/html; charset=UTF-8">
<title>Login Page</title>
</head>
<body>

<h1>Login Page</h1>
<form method="POST" action="LoginController">
                Username: <input type="text" name="username">
                <br>
                Password: <input type="password" name="password">
                <br>
                <input type="submit" value="submit">
</form>
<a href="NewAccount2.jsp">Make new account</a>
<br/>
Account Creation Successful!
</body>
</html>
```

```
        %>

</body>
</html>
```

Appendix C: Data

Table 2: Modulus Data gathered from Stress-Strain data.

Material	Modulus
2% Silk	1.3313
2% Silk	1.4402
2% Silk	1.4052
2% Silk	1.5314
3% Silk	1.6866
3% Silk	1.5321
3% Silk	1.4143
3% Silk	1.5790
4% Silk	3.0030
4% Silk	3.8436
4% Silk	3.4034
4% Silk	2.0478
4% Silk	3.6063
4% Silk	2.1475
5% Silk	2.4919
5% Silk	1.1807
5% Silk	4.0005
5% Silk	3.1921
5% Silk	1.6560
5% Silk	3.8830
6% Silk	1.9617
6% Silk	1.9985
6% Silk	2.4922
6% Silk	3.3014
6% Silk	2.2309
6% Silk	1.8677
6% Silk	1.1195
6% Silk	2.6976

Appendix D: Notes File

Source Title	Targeting cancer cell metabolism in pancreatic adenocarcinoma
Citation	Cohen, R., Neuzillet, C., Tijeras-Raballand, A., Faivre, S., de Gramont, A., & Raymond, E. (2015). Targeting cancer cell metabolism in pancreatic adenocarcinoma. *Oncotarget*, *6*(19), 16832–16847.
Found By:	Looking up "cancer cell targeting" on WPI Summons
Type	Journal
Keywords	glutamine; glycolysis; hypoxia; metformin; warburg effect
Summary	PDAC is a very difficult to treat cancer and is predicted to be the second leading cause of cancer death by 2030. Because of their rough metabolic conditions, metabolic reprogramming was found to be the best way to solve this problem. The easiest way to metabolically program was through cell targeting
Reason for interest	I am really interested in targeted therapy and more efficient ways to implement it
Notes	• PDAC is the 5th leading cause of cancer related deaths • By 2030 it will be second leading cause of cancer death • Highly invasive with early metastatic potential • Hard to treat • All treatments are hardly successful and new treatments are being researched • Cancer cells need lots of ATP and macromolecules • Metabolic targeting is highly effective because cancer cells rely on their metabolism

Source Title IL-22, cell regeneration and autoimmunity

Citation	Koopour, E., Bellemore, S. M., & Singh, B. (2014). IL-22, cell regeneration and autoimmunity. *Science Direct*, *74*(1), 35-42. Retrieved September 17, 2017.
Found By:	Looking up "cell regeneration" on WPI Summons
Type	Journal
Keywords	Aryl hydrocarbon receptor (AhR); Autoimmune diseases; IL-22; IL-23; Regenerating (Reg) genes
Summary	IL-22 is a cytokine that has both pro and anti-inflammatory feature. Both of these result in a heightened cellular regeneration capability, and researchers tried to harness this ability to help tissue regeneration
Reason for interest	I am really interested in cellular regeneration
Notes	• IL-22 is a cytokine produced by the T-cell in humans • It is also found in mice with a 79% homology • Produced by mainly TH-17 cells

Source Title	Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells
Citation	Diekman, B. O., Christoforou, N., Willard, V. P., Sun, H., Sanchez-Adams, J., Leong, K. W., & Guilak, F. (2012). Cartilage tissue engineering using differentiated and purified induced pluripotent stem cells. Proceedings of the National Academy of Sciences of the United States of America, 109(47), 19172–19177. http://doi.org/10.1073/pnas.1210422109
Found By:	Looking up "Jeannine Coburn Papers" on Google
Type	Journal Article
Keywords	chondrocyte, bone morphogenetic proteins, transforming growth factor-beta, cartilage micromechanics, cartilage regeneration
Summary	iPSCs are used for the regeneration of focal cartilage damage
Reason for interest	I am really interested in cellular regeneration
Notes	• Focal cartilage damage can lead to osteoarthritis • Articular cartilage does not regenerate quickly • Current procedures for treating focal cartilage damage are very risky/ may not work perfectly • Induced pluripotent stem cells are non-invasive and can be programmed for a variety of uses • One issue with iPSC's is the unequal distribution of cell differentiation • Goal was to create engineered tissue from iPSC's by using predifferentiated cells • Researchers found that with the correct differentiation protocols, iPSCs can quickly produce cartilage

Source Title	Biomaterials & scaffolds for tissue engineering
Citation	O'brien, F. J. (2011). Biomaterials & scaffolds for tissue engineering. *Materials today*, *14*(3), 88-95.
Found By:	Looking up “scaffolds for tissue engineering” on google
Type	Review
Keywords	Biomaterials, tissue engineering, natural polymers, synthetic polymers
Summary	This article goes over various different types of scaffolds and properties that are important for scaffolding of cells in different parts of the body
Reason for interest	I am really interested in cellular regeneration

Notes	• Scaffolds are 3d porous substances that give the cells a backbone to grown on
	• Scaffolds have certain criteria that they must fill
	  ○ Biocompatibility
	  ○ Biodegradability
	  ○ Mechanical Properties
	  ○ Scaffold Architecture
	  ○ Manufacturing Process
	• 3 main types of biomaterials
	  ○ Ceramics
	  ○ Synthetic Polymers
	  ○ Natural Polymers
	• Each group has its own advantages and disadvantages, so generally multiple types are used for composite
	• Ceramic scaffolds are generally used for bone tissue regeneration because of the low elasticity

Source Title	Challenges in tissue engineering
Citation	Ikada, Y. (2006). Challenges in tissue engineering. *Journal of the Royal Society Interface*, *3*(10), 589–601. http://doi.org/10.1098/rsif.2006.0124
Found By:	Looking up "problems with cell regeneration" on google
Type	Review
Keywords	cell source; scaffold; growth factor; carrier; animal studies; human trials
Summary	This review went over the different aspects of tissue engineering and the difficulties and challenged of each of those parts
Reason for interest	I am really interested in cellular regeneration

Notes	• When tissues are damaged, the first choice to reconstruct organs is generally organ transplants
	• Problems with transplants include shortages of donated organs, immune rejection
	• Tissue engineering was introduced 30 years ago as a solution to the biofunctionality and biocompatibility issues
	• Tissue engineering uses three basics tools
	  ○ Cells
	  ▪ Autologous cells- patients own
	  ▪ Allogenic cells- human other than patients cells
	  ▪ Xenogenic cells- animal
	  ▪ Autologous cells are the main use of tissue
	  ○ Scaffolds
	  ○ Growth Factor
	• The three tools aren't always used for tissue engineering- for example, dermal tissue only needs a scaffold

Source Title	An Overview of Various Biomimetic Scaffolds: Challenges and Applications in Tissue Engineering
Citation	Nigam R, Mahanta B (2014) An Overview of Various Biomimetic Scaffolds: Challenges and Applications in Tissue Engineering. J Tissue Sci Eng 5:137. doi:10.4172/2157-7552.1000137
Found By:	Looking up "problems with cell scaffolding" on google
Type	Review
Keywords	Biomaterial; Tissue engineering; Scaffold; Biodegradable; Biomimetic; Regeneration
Summary	This review goes over different biomaterials and scaffolds and the advantages that each has on tissue engineering
Reason for interest	I am really interested in cellular regeneration

Notes	• General strategies of tissue engineering are:
	  ○ Implantation of constructs reinforced with the target cells into the organism
	  ○ Delivery of growth factors to the constructs
	  ○ Adhesion of cells with the surrounding tissues.
	• In vivo, cells have an ECM to guide their growth
	• In tissue engineering, the role of the ECM is taken by scaffolds
	• Biomimetic scaffolds have the best potential
	• Natural polymers are generally biocompatible but have poor physical and mechanical properties while synthetic polymers have low biocompatibility but perfect physical and mechanical properties for any designated area
	• Three main ways of creating biomimetic scaffolds
	  ○ Porogens in biomaterials: particles are dissolved into solvents and the solution is then hardened. Particles are then cleansed out creating porous structures
	  ○ Rapid prototyping technologies- technologies that can create scaffolds
	  ○ Woven or non-woven fibers
	• Applications for living longer lives, looking younger for longer
	• Types of scaffolds:
	  ○ Porous scaffolds: scaffolds that have interconnected porous systems, good for making artificial blood vessels, peripheral never growth and soft tissue engineering
	  ○ Hydrogel scaffolds
	  ○ Nanofibers: the high porosity and surface area mimics real ECM's
	  ○ Acellular- scaffolds that are created by removing the living cells from a preexisting ECM

Source Title	Nanofibers and their applications in tissue engineering
Citation	Vasita, R., & Katti, D. S. (2006). Nanofibers and their applications in tissue engineering. *International Journal of Nanomedicine*, *1*(1), 15–30.
Found By:	Looking up “nanofibers for cell regeneration” on google
Type	Review
Keywords	electrospinning, phase separation, self-assembly, nanofiber, biomaterial, tissue engineering, scaffold, drug delivery
Summary	This article goes into great detail about nanofibers and the role they play in cellular scaffolds
Reason for interest	I am really interested in cellular regeneration

Notes	• Nanofibers have been researched and are one of the best methods of for scaffolds because of the high surface area to volume ratio
	• Nanofibers microporous structure favors adhesion, proliferation, migration and differentiation
	• There are three main methods of creating nanofibers
	  ○ Electrospinning
	  ▪ Electrospinning can create ultra-thin fibers
	  ▪ A polymeric solution is given an electric potential
	  ▪ The solution is then put at the tip of a needle , with surface tension keeping it together
	  ▪ The electric potential is then increased crating a cone called the Taylor Cone
	  ▪ A spinning collector then collects the strands
	  ▪ By changing the design of the collecting electrode, the alignment and assembly of nanofibers can be changed
	  ○ Self-assembly
	  ○ Phase separation
	  ▪ 5 main steps
	  • Dissolution of the polymer
	  • Liquid-liquid phase separation process
	  • Polymer gelation
	  • Extraction of solvent from the gel
	  • Freezing and freeze drying under vacuum
	  ▪ This process gets very close to natural ECM’s
	  ▪ Many different factors affect the scaffold from this, making it very difficult to perfect
	• Biomaterials:
	  ○ Collagen- very biocompatible, natural polymer
	  ○ Chitosan- waterproof, natural polymer, enhanced attachment of osteoblasts
	  ○ Hyaluronic acid- a component of natural ECM

Source Title	Scaffolding in tissue engineering: general approaches and tissue-specific considerations
Citation	ıan, B. P., & Leong, K. W. (2008). Scaffolding in tissue engineering: general approaches and tissue-specific considerations. *European Spine Journal*, *17*(Suppl 4), 467–479. http://doi.org/10.1007/s00586-008-0745-3
Found By:	Looking up “cell scaffolds” on google
Type	Review
Keywords	Tissue engineering, Scaffolding, Scaffolds, Biomaterials, Intervertebral disc
Summary	This article is a general overview of the different aspects important when creating a biological scaffold
Reason for interest	I am really interested in cellular regeneration

Notes	• All cells except for blood cells are dependent on ECM’s
	• 5 main functions of ECMs
	  ○ ECMs provide structural support for the cells to grow on
	  ○ ECMs give the tissue the rigidity and flexibility required for its basic processes
	  ○ ECMs provide the cues for specific cell functions
	  ○ ECMs act as reservoirs of growth factors
	  ○ ECMs provide a degradable physical environment which allows for morphological changes
	• Important aspects when creating artificial ECMs
	  ○ Architecture- the scaffold
	  ○ Tissue compatibility
	  ○ Bioactivity
	  ○ Mechanical property
	• Cell seeding has become one of the biggest areas of research in cell scaffolding
	• Natural biomaterials generally have good biocompatibility but poor mechanical properties
	• Synthetic biomaterials are the exact opposite- they have good mechanical properties but poor biocompatibility.
	• Acellular ECMs
	  ○ This process removes all of the donating animals cells, leaving an ECM with similar materials to the receiver
	  ○ First, cells are lysed through a freeze thawing or submerging in ionic solutions
	  ○ Then, cellular components are removed with a trypsin or

Source Title	Polymeric Scaffolds in Tissue Engineering Application: A Review
Citation	Brahatheeswaran Dhandayuthapani, Yasuhiko Yoshida, Toru Maekawa, and D. Sakthi Kumar, "Polymeric Scaffolds in Tissue Engineering Application: A Review," International Journal of Polymer Science, vol. 2011, Article ID 290602, 19 pages, 2011. doi:10.1155/2011/290602
Found By:	Looking up "biomaterials for cell scaffolding" on google
Type	Review
Keywords	Tissue engineering, Scaffolding, Scaffolds, Biomaterials, Intervertebral disc
Summary	By using a gamma secretase inhibitor, the researchers were able to regenerate hair cells in mammals, which are unable to regenerate hair cells on their own
Reason for interest	I am really interested in cellular regeneration

Notes	• Scaffolds have four main functions
	  ○ Promote cell biomaterial interactions
	  ○ Permits sufficient transports of gases and nutrients
	  ○ Biodegrade at a controllable rate
	  ○ Promote a minimal degree of inflammation
	• Natural polymers were the first polymers used in a tissue engineering setting
	• Synthetic polymers, especially PLA, PGA, and PLGA, are also used
	• Bioactive ceramics, such as HAP and TCP are used for hard tissue engineering
	• Although effective solutions already exist, a method to control pore size must be made in order to successfully create artificial blood vessels.
	• Six main ways of categorizing different scaffolds
	  ○ External geometry
	  ○ Surface properties
	  ○ Porosity and pore size
	  ○ Interface adherence
	  ○ Degradation characterization
	  ○ Mechanical competence
	• Different mechanical properties governing scaffold similarity to natural ECMs
	  ○ Elastic modulus
	  ○ Flexural modulus
	  ○ Tensile strength
	  ○ Maximum strain

Source Title	Lyophilized Silk Sponges: A Versatile Biomaterial Platform for Soft Tissue Engineering
Citation	Rnjak-Kovacina, J., Wray, L. S., & Burky, K. A. (2015). Lyophilized Silk Sponges: A Versatile Biomaterial Platform for Soft Tissue Engineering. ACS Publications, 260-270. doi:10.1021/ab500149p
Found By:	Looking up "Silk Lyophilization" on google
Type	Journal
Keywords	silk, scaffold, soft tissue engineering, lyophilized sponge, biomaterial
Summary	This article gave an overview of silk lyophilization
Reason for interest	My project is on cellular scaffolds

Notes	• There are many different types of soft tissue damage
	  ○ Muscle
	  ○ Skin
	  ○ Adipose
	  ○ Neural
	• Soft tissue scaffolds need to be pliable to match contours of the tissue
	• Pore size should generally be between 100 and 300 micrometers
	• Most biomaterials used for soft tissue regeneration are synthetic polymers such as polypropylene
	• Silk has been a growing soft tissue biomaterial because of its high compatibility and degradability
	• Silks can also be made into porous scaffolds, sponges, hydrogels, films, fibers and microspheres
	• Different methods of silk scaffold synthesis have been used, such as salt leaching, gas foaming and lyophilization
	• Of these methods, salt leaching has been used because of its potential for bone tissue regeneration
	• Lyophilization process
	  ○ Silk is degummed to remove sericin. Based on time spent degumming, molecular weights of silk proteins are changed
	  ○ Aqueous silk solutions are put into wells of a well plate and placed into a lyophilizer
	  ○ The dry scaffolds are then removed from the molds and put into an autoclave to insolubilize the scaffolds
	  ○ Once insolubilized,

Source Title	An overview of tissue and whole organ decellularization processes
Citation	Crapo, P. M., Gilbert, T. W., & Badylak, S. F. (2011). An overview of tissue and whole organ decellularization processes. *Biomaterials*, *32*(12), 3233-3243.
Found By:	Looking up "Silk Lyophilization" on google
Type	Journal
Keywords	extracellular matrix, biomaterials, scaffolds, decellularization, regenerative medicine
Summary	This article gave an overview of silk lyophilization
Reason for interest	My project is on cellular scaffolds
Notes	○ Scaffolds composed of natural ECM are being used for a variety of reconstructive surgical applications ○ ECMs have a microadaptable structure ○ Natural ECMs have been shown to influence cell mitogenesis and chemotaxis ○ Decellularization: ▪ Decellularizing agents for each tissue depends primarily on the properties of that tissue ▪ Using improper decellularizing agents can alter the properties of the ECM ▪ Three main types of chemical agents are used: chemical agents, enzymatic agents and physical agents ○ Chemical agents ▪ Acids and bases • Cause hydrolytic degradation of biomolecules ▪ Hypotonic and hypertonic solutions • Causes cell lysis and detachment from ECM ▪ Detergents • Dissolve cell membranes and dissociate DNA from proteins ▪ Alcohols ○ Biologic agents- complete cell removal is fairly difficult, but they may add in the removal of portions of the cellularized tissue ○ Physical agents ▪ Temperature- Freeze-thaw cycles effectively lyse the cells, but intercellular components remain ▪ Force and pressure- dissociate cells through abrasion